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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
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EXAMINER	
CLOW, LORI A	

ART UNIT	PAPER NUMBER
1631	

DATE MAILED: 09/28/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/891,517	KURANE ET AL.	
	Examiner	Art Unit	
	Lori A. Clow, Ph.D.	1631	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *109-149* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *109-149* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Applicants' response, filed 10 July 2006, has been fully considered. Rejections and/or objections not reiterated from previous office actions are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set presently being applied to the instant application.

Claims 109-149 are currently pending. Claims 1-108 have been cancelled. Claims 109-149 are newly added.

Claim Objections

Claims 141 and 143 are objected to because of the following informalities: Claim 141 recites "fragments obtaining by said digestion". This should read, "fragments obtained by said digestion".

Claim 143 recites at step (3) "the obtained gene fragments is thermally modified". This is awkward claim language and should be corrected to read, "thermally modifying the obtained gene fragments into single-stranded forms"

Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 109-149 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 109 and 110 recite the following steps: (1) amplifying a target gene and monitoring the amplification by real-time PCR; (2) performing a polymorphous analysis selected from the group consisting of T-RELP, RFLP, SSCP, or CFLP with respect to the amplified target gene to determine a polymorphous composition ratio of individual species of the target gene; (3) determining the initial amount of the target gene; and (4) determining the initial amounts of individual species of the target gene.

Claims 109, 110, and 143 are rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: the relationship between the amplification step, the polymorphous analysis step, and the determination of initial amounts of target and species. For example, what method is employed for the determination of the initial amount of the target gene or the determination of the initial amount of individual species of the target? Without the recitation of the quencher probe in the amplification step of the target, it is unclear what is being measured to determine amounts. Further, it is unclear whether the "polymorphous composition ratio of the individual species" is what is being employed to measure the amounts of the target versus the amounts of the individual species. Clarification is requested.

Claims 138 and 145 recite, "wherein said T-REFP is an analysis". It is unclear is this intended to be and active method step in addition to the T-RELP analysis of claims 137 and 144, respectively or some other limitation. Clarification is requested.

Further, it is unclear if the T-REFP and T-RELP are two different analysis methods or if there is simply a typographical error in the claims. There is insufficient antecedent basis in claims 138 and 145 for T-REFP. Clarification is requested.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* v. *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 109, 112-114, and 137-142 are rejected under 35 U.S.C. 103(a) as being obvious over 6,699,661 B1 (Kurane et al.), in view of Liu et al. (Applied and Environmental Microbiology (1997) November, pages 4516-4522).

The applied reference has a common inventor with the instant application. Based upon the earlier effective U.S. filing date of the reference, it constitutes prior art only under 35 U.S.C. 102(e). This rejection under 35 U.S.C. 103(a) might be overcome by: (1) a showing under 37 CFR 1.132 that any invention disclosed but not claimed in the reference was derived from the inventor of this application and is thus not an invention "by another"; (2) a showing of a date of invention for the claimed subject matter of the application which corresponds to subject matter disclosed but not claimed in the reference, prior to the effective U.S. filing date of the reference under 37 CFR 1.131; or (3) an oath or declaration under 37 CFR 1.130 stating that the application and reference are currently owned by the same party and that the inventor named in the application is the prior inventor under 35 U.S.C. 104, together with a terminal disclaimer in accordance with 37 CFR 1.321(c). This rejection might also be overcome by showing that the reference is disqualified under 35 U.S.C. 103(c) as prior art in a rejection under 35 U.S.C. 103(a). See MPEP § 706.02(l)(1) and § 706.02(l)(2).

The instant claims are drawn to a method of determining the initial amounts of individual species of a target gene.

With respect to claim 109, Kurane teaches a method for determining a concentration of a target nucleic acid by using a nucleic acid probe labeled with a fluorescent dye (abstract) wherein (a) a target gene is amplified and monitored by real-time PCR (column 5, lines 5-7; column 18, line 17); (b) polymorphism analysis (column 8, lines 53-54; column 12, lines 20-52);

(c) determining target amount (column 8, lines 59-61); and (d) determining species amount (column 9, lines 30-35).

With respect to claim 112, Kurane teaches that the probe is end labeled with a fluorescent dye (column 9, lines 43-50); the probe hybridizes at the end portion of the target where at least on G (guanine) base exists in a base sequence of the target at a position 1 to 3 bases apart from an end base of the target (column 11, lines 14-18; column 19, lines 2-11); and the fluorescent dye is reduced in fluorescence emission when the probe hybridizes to the target (column 15, lines 61-64).

With respect to claim 113, Kurane teaches that the end labeled probe has a sequence designed such that when the probe hybridizes to the target, plural base pairs in a probe-nucleic acid complex form at least one G (guanine) and C (cytosine) pair (column 11, lines 19-21) and that fluorescence is reduced.

With respect to claim 114, Kurane teaches a nucleic acid probe wherein the probe is labeled with a fluorescent dye and is modified on the 3' and 5' end (column 19, lines 12-32) and become one pair at the modification portion (column 19, lines 39-45).

With respect to claim 142, Kurane teaches that the nucleic acid probe is labeled with fluorescent dye at the 5'-end and that the dye is selected from FITC, Texas red, 6-Joe, TMR, Alexa 488, Alexa 532, "BODIPY FL/C3", and "BODIPY FL/C6" (column 9, lines 43-67 to column 10, lines 1-5).

With regard to the step of performing a polymorphous analysis, Kurane does not specifically teach that the analysis is selected from the group consisting of T-RELP, RFLP, SSCP, or CFLP.

However, Liu teaches a quantitative molecular technique employing PCR in which one of two primers used was fluorescently labeled at the 5' end in which the amplification products were digested with restriction enzymes and the fluorescently labeled terminal restriction fragment was precisely measured using an automated sequencer.

In regard to claims 109 and 137 the method employed for analysis of the PCR product was terminal restriction fragment length polymorphism (T-RFLP in Liu et al., which is equivalent to the T-RELP method of the instant invention) (abstract).

In regard to claim 138, Liu teaches digestion using an endonuclease selected from Bso, FI, Hha, Hph I, Mn1 I, Alu I, and Msp I (page 4517, column 1, Material and Methods).

In regard to claim 139, Liu teaches the restriction endonuclease from Rca, Alu I, or Msp I (page 4517, column 1, Material and Methods).

In regard to claim 140, Liu teaches digestion using an endonuclease Hha (page 4517, column 1, Material and Methods).

In regard to claim 141, Liu teaches that the gene fragments are precisely measured by an automated sequencer (abstract; page 4517, column 2, Material and Methods).

It would have been prima facie obvious to one of ordinary skill in that art at the time of the invention to have combined the fragment analysis technique using T-RFLP of Liu in the method for determining a concentration of a target nucleic acid by using a nucleic acid probe labeled with a fluorescent dye of Kurane. One would have been motivated to do so with an expectation of success because Kurane states that the invention is for the determination of polymorphism or mutation of a target nucleic acid or gene, whereby it is possible to analyze or determine polymorphism of a target nucleic acid by hybridizing the probe to the target and

measuring the emission intensity (column 12, lines 20-33). Liu motivates one to combine the labeling method with the restriction technique of T-RFLP to provide a sensitive and rapid means to assess diversity and obtain a distinctive fingerprint of individuals in a microbial community (page 4517, column 1).

Claims 110, 111, 116-136, 144, and 149 are rejected under 35 U.S.C. 103(a) as being unpatentable over 6,699,661 B1 (Kurane et al.), in view of US 6,727,356 B1 (Reed et al.).

The instant claims are drawn to a method of determining the initial amounts of individual species of a target gene.

In regard to claims 110 and 111 Kurane teaches a method for determining a concentration of a target nucleic acid by using a nucleic acid probe labeled with a fluorescent dye (abstract) wherein (a) a target gene is amplified and monitored by real-time PCR (column 5, lines 5-7; column 18, line 17); (b) polymorphism analysis (column 8, lines 53-54; column 12, lines 20-52); (c) determining target amount (column 8, lines 59-61); and (d) determining species amount (column 9, lines 30-35).

In regard to claim 120 and 121, Kurane teaches that the probe is labeled on the 3' end (column 19, lines 12-32)

In regard to claims 122 and 123, Kurane teaches that the probe is labeled on the 5' end (column 19, lines 12-32).

In regard to claim 124, Kurane teaches that the probe has a G or C as a 5' end base and is labeled at the 5' end (column 19, lines 1-32).

In regard to claims 125 and 126, Kurane teaches that the hydroxyl group has been phosphorylated (column 11, lines 33-64).

In regard to claims 127 and 128, Kurane teaches that the probe is labeled at a 5' end phosphate group and/or a 3' end phosphate group (column 11, lines 33-64).

In regard to claims 129 and 130, Kurane teaches that the oligonucleotide is chemically modified (column 10, line 17).

In regard to claims 131 and 132, Kurane teaches that the modification is 2'-O-methyloligonucleotide (column 10, line 18).

In regard to claims 133-136, Kurane teaches that the chimeric oligonucleotide is 2'-O-methyloligonucleotide (column 10, lines 6-22).

In regard to claim 149, Kurane teaches that the nucleic acid probe is labeled with fluorescent dye at the 5'-end and that the dye is selected from FITC, Texas red, 6-Joe, TMR, Alexa 488, Alexa 532, "BODIPY FL/C3", and "BODIPY FL/C6" (column 9, lines 43-67 to column 10, lines 1-5).

Kurane does not specifically teach that the probe is labeled with a fluorescent dye and a quencher substance, wherein the intensity of the fluorescence in a hybridization reaction increases when the probe hybridizes with the target.

However, in regard to claims 110 and 111, Reed teaches fluorescent quenching detection reagents and methods (abstract). In particular, Reed discloses a quencher for use in analytical methods, such a FRET analysis in which fluorophores are attached at 5' and 3' positions (column 5, lines 4-16). The method is directed to detection of PCR-generated nucleic acid sequences in which one or more fluorescent oligonucleotide conjugates are used as probes to identify target

nucleic acids by assaying hybridization between probes and the target (column 36, lines 23-27). In the method, a probe containing both a fluorescent label and a quenching agent, which quenches fluorescent emission of the fluorescent label, is employed (column 37, lines 27-29). Further, subsequent to hybridization of the fluorophore/quencher-labeled probe to its target, it becomes a substrate for the exonucleolytic activity of a polymerizing enzyme which has initiated polymerization at an upstream primer. Exonucleolytic degradation of the probe releases a fluorescent label form the probe, and hence from the vicinity of the quenching agent, allowing detection of a fluorescent agent (column 37, lines 35-42).

In regard to claims 116 and 117, Reed teaches that the oligonucleotide is labeled on the same side with the fluorescent dye and quencher (column 15, lines 23-25).

In regard to claims 118 and 119, reed teaches that the distance between bases is 1 to 20 bases (column 26, line 42).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to has combined the quencher probes of Reed in the method of Kurane, for the detection of polymorphisms. One would have been motivated to do so because Kurane teaches that the method of the invention is appropriate for measuring the change in intensity of fluorescence corresponding to the concentration of target nucleic acids, therefore the target may be quantitated from that change (column 14, lines 14-26).

Claims 115 and 143-148 are rejected under 35 U.S.C. 103(a) as being unpatentable over 6,699,661 B1 (Kurane et al.), in view of US 6,727,356 B1 (Reed et al.), as applied to claims 110

and 11 above, in further view of Liu et al. (Applied and Environmental Microbiology (1997) November, pages 4516-4522).

As stated above, Kurane teaches a method for determining a concentration of a target nucleic acid by using a nucleic acid probe labeled with a fluorescent dye (abstract) wherein (a) a target gene is amplified and monitored by real-time PCR (column 5, lines 5-7; column 18, line 17); (b) polymorphism analysis (column 8, lines 53-54; column 12, lines 20-52); (c) determining target amount (column 8, lines 59-61); and (d) determining species amount (column 9, lines 30-35).

Reed teaches a method whereby fluorescent quenching detection reagents and methods are employed (abstract). In particular, Reed discloses a quencher for use in analytical methods, such a FRET analysis in which fluorophores are attached at 5' and 3' positions (column 5, lines 4-16). The method is directed to detection of PCR-generated nucleic acid sequences in which one or more fluorescent oligonucleotide conjugates are used as probes to identify target nucleic acids by assaying hybridization between probes and the target (column 36, lines 23-27). In the method, a probe containing both a fluorescent label and a quenching agent, which quenches fluorescent emission of the fluorescent label, is employed (column 37, lines 27-29). Further, subsequent to hybridization of the fluorophore/quencher-labeled probe to its target, it becomes a substrate for the exonucleolytic activity of a polymerizing enzyme which has initiated polymerization at an upstream primer. Exonucleolytic degradation of the probe releases a fluorescent label form the probe, and hence from the vicinity of the quenching agent, allowing detection of a fluorescent agent (column 37, lines 35-42).

Neither Kurane nor Reed teach that the gene fragments are detected by a sequencer, as in claims 143 and 148. However, Liu et al. teach a method of polymorphism detection in which terminal restriction fragments are precisely measured by an automated DNA sequencer (page 4516: abstract; page 4517, column 2, GenScan system, which measures fluorescent intensity).

In regard to claims 115 and 144, Liu teaches that the method is T-RFLP (abstract).

In regard to claim 145, Liu teaches digestion using an endonuclease selected from Bso, FI, Hha, Hph I, Mnl I, Alu I, and Msp I (page 4517, column 1, Material and Methods)

In regard to claim 146, Liu teaches the restriction endonuclease from Rca, Alu I, or Msp I (page 4517, column 1, Material and Methods).

In regard to claim 147, Liu teaches digestion using an endonuclease Hha (page 4517, column 1, Material and Methods).

It would have been prima facie obvious to one of ordinary skill in the art at the time of the invention to have combined the methods of Kurane and Reed with the T-RFLP method and fluorescent detection of Liu. One would have been motivated to do so because Kurane teaches that the method of the invention is appropriate for measuring the change in intensity of fluorescence corresponding to the concentration of target nucleic acids, therefore the target may be quantitated from that change (column 14, lines 14-26). Further, the invention is for the determination of polymorphism or mutation of a target nucleic acid or gene, whereby it is possible to analyze or determine polymorphism of a target nucleic acid by hybridizing the probe to the target and measuring the emission intensity (column 12, lines 20-33).

No claims are allowed.

Inquiries

Papers related to this application may be submitted to Technical Center 1600 by facsimile transmission. Papers should be faxed to Technical Center 1600 via the PTO Fax Center. The faxing of such papers must conform with the notices published in the Official Gazette, 1096 OG 30 (November 15, 1988), 1156 OG 61 (November 16, 1993), and 1157 OG 94 (December 28, 1993) (See 37 CFR § 1.6(d)). The Central Fax Center Number is (571) 273-8300.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lori A. Clow, Ph.D., whose telephone number is (571) 272-0715. The examiner can normally be reached on Monday-Friday from 10 am to 6:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Andrew Wang can be reached on (571) 272-0811.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

September 22, 2006
Lori A. Clow, Ph.D.
Art Unit 1631
Lori A. Clow
Patent Examiner